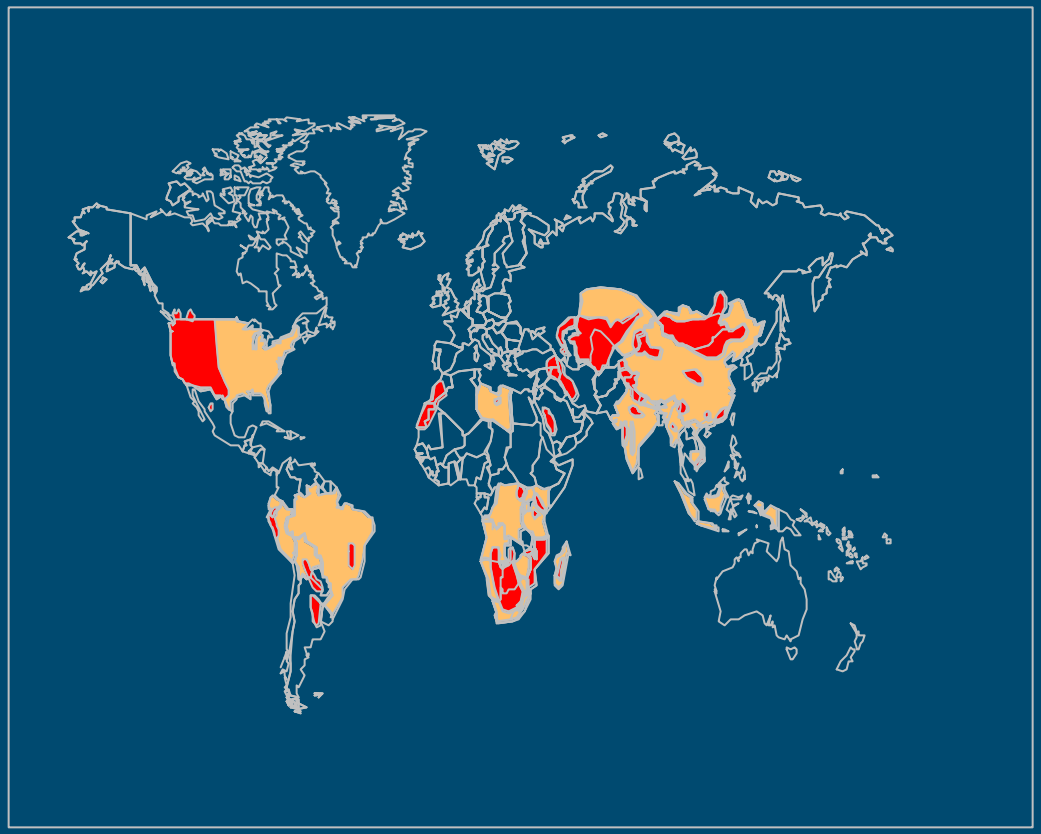
Vaccine design and Rationale

Richard W Titball

Worldwide incidence of plague



- Compiled from WHO,CDC, and country sources

Countries reporting plague,1970-1995

Probable foci

[dstl]

29 November 2004

Dstl is part of the MOD

Y. pestis is a biowarfare and bioterrorism agent

PAGE 10 CHRONICLE of the FUTURE

Bio-bombers strike in New York

20.02.13 **THE DEATH TOLL following the biological weapons attack on New York yesterday is expected to reach 20,000.**

Three unidentified remote-controlled unmanned air vehicles (UAVs) sprayed Manhattan with a biological agent during the morning rush hour, as part of a co-ordinated onslaught that brought the city's financial quarter to a standstill.

The city's biological weapons sensor systems – installed after the failed attack on St Louis in 2008 – were disrupted because the agent was micro-encapsulated in an unknown substance that interfered with the multiplex (DNA) sensor chips. Dense smoke from the fire at the Empire State Building also affected the sensors. Sabotage of the city's electrical supply and signal systems left commuters trapped for long periods before they could be told to put on their face masks to avoid breathing in the agent.

Wall Street closed, throwing the world's financial markets into chaos. There seems little hope of the stock exchange reopening immediately.

Fear of further attacks on the city has led to the president, George Bush Jr, being evacuated to Camp David. From there he broadcast the warning that America was prepared to use its most advanced weapons against those responsible for the attack.

The Warfare Research Institute in Maryland is understood to have identified the agent involved. Unofficial sources suggest that it is a variant of the normally benign tularemia *Francisella tularensis*, genetically engineered to make it resistant to multiple antibiotics, and able to rapidly produce toxic chemicals as it multiplies in the bodies of victims.

It is thought the perpetrators come from the Middle East, where the crisis over who controls the oil reserves of the former Iraq is expected to prompt further military action by the United Nations Worldwide Intervention Force. Sub-national groups in the war-torn region certainly possess the biotechnology required to produce the tularemia agent.

America considered retaliating with cruise missile strikes, but was advised against hasty action by the European Union's Peace Institute. The institute, which was set up after the nuclear exchanges of the early years of the century, warned that there are many other resentful groups with the equivalent scientific expertise. Action against the wrong group could invite further terrorism. MD

Populations in which plague vaccines might be used

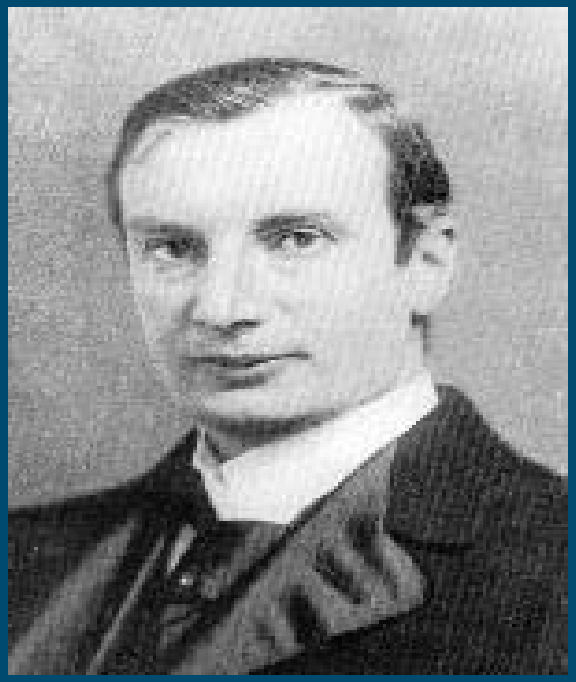
- Researchers and laboratory personnel working with virulent strains of *Y. pestis*.
- In military personnel, field workers and agricultural consultants who move into areas of the world where plague is endemic
- In indigenous populations, where there is recurrent or intense plague activity
- In individuals who might come into contact with animals in areas where enzootic plague foci are present
- In military populations who might be exposed to *Y. pestis* as a bioweapon
- In civilian populations who might be exposed to *Y. pestis* as a bioterrorist weapon

Existing vaccines

- **Killed whole cell vaccine**
  - multiple doses over 6 months
  - used in some at risk populations in the West
- **live attenuated vaccine (EV76)**
  - used mainly in fSU and Madagascar
  - not licensed in Europe/USA

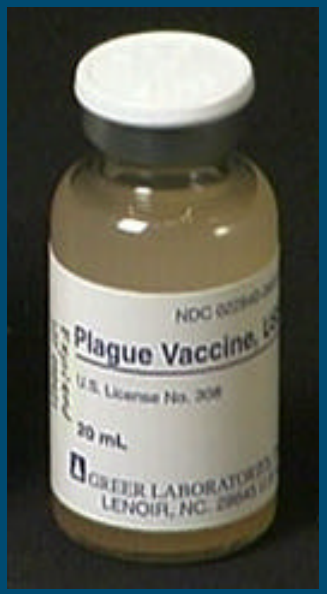
Killed whole cells vaccines

- Plague outbreak in Bombay, 1896
- Waldemar Haffkine tests a KWC vaccine in 1897
- On himself !!



Iterations of KWC vaccines

- Haffkine vaccine 1879
- The “Army Vaccine” 1946
- Cutter vaccine (USA) -1994
- Greer vaccine (USA) 1994-1999
- CSL vaccine (Australia) currently available



- Heat or formaldehyde killed bacteria

Immunisation schedules
(for adults)

Primary course

Booster

Greer

1-3 months

6 months

6 months

CSL vaccine

1-4 weeks

6 months

Evidence that KWC vaccines work

- Protection against *Y. pestis* in animals

- Efficacy data from US servicemen in Vietnam
  - Incidence of plague in Vietnamese; 333 cases / 10^6 person years
  - Incidence of plague in vaccinated servicepersons; 1 case / 10^6 person

  - BUT the incidence of murine typhus (also spread by *X. cheopis*) similar

The mouse protection test

Vaccinee serum (0.5ml)

Mouse

G. Pig

monkey

human

Groups of 10 mice

s.c challenge

100 MLD

Y. pestis

Mouse Protection index (MPI)

% mortality over 14 days

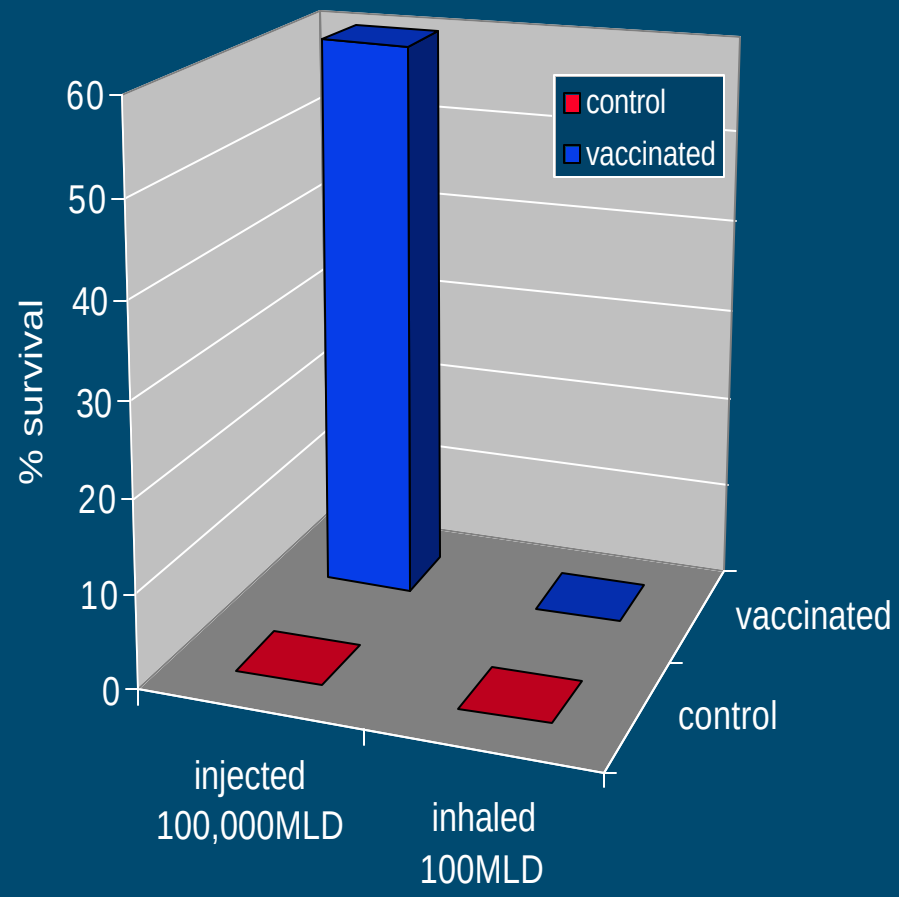
Average time to death

MPI < 10 = acceptable

MPI test previously approved by the U.S. Public Health Service for the testing of plague vaccines

Evidence that KWC vaccines don't work

- Pneumonic plague reported in vaccinated individuals
- experimental studies in animals

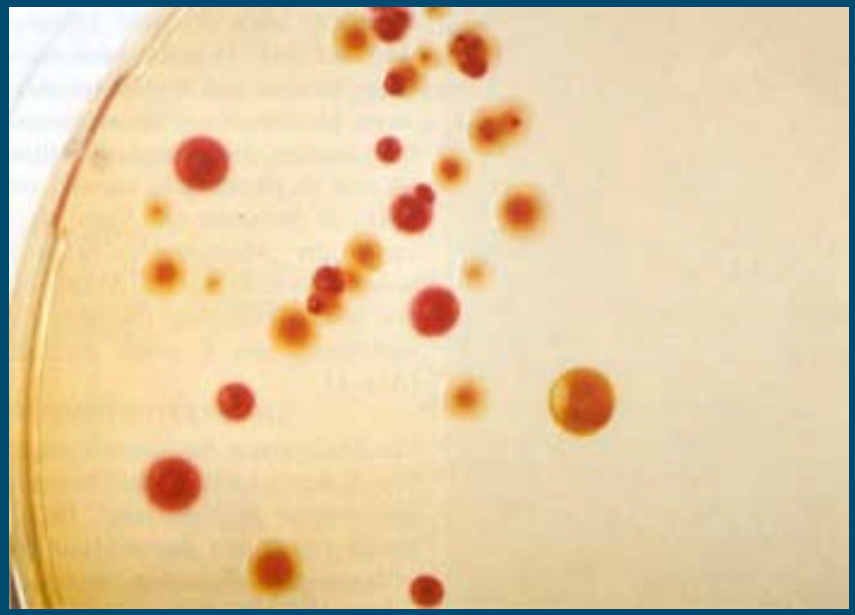


Reactogenicity of Greer vaccine

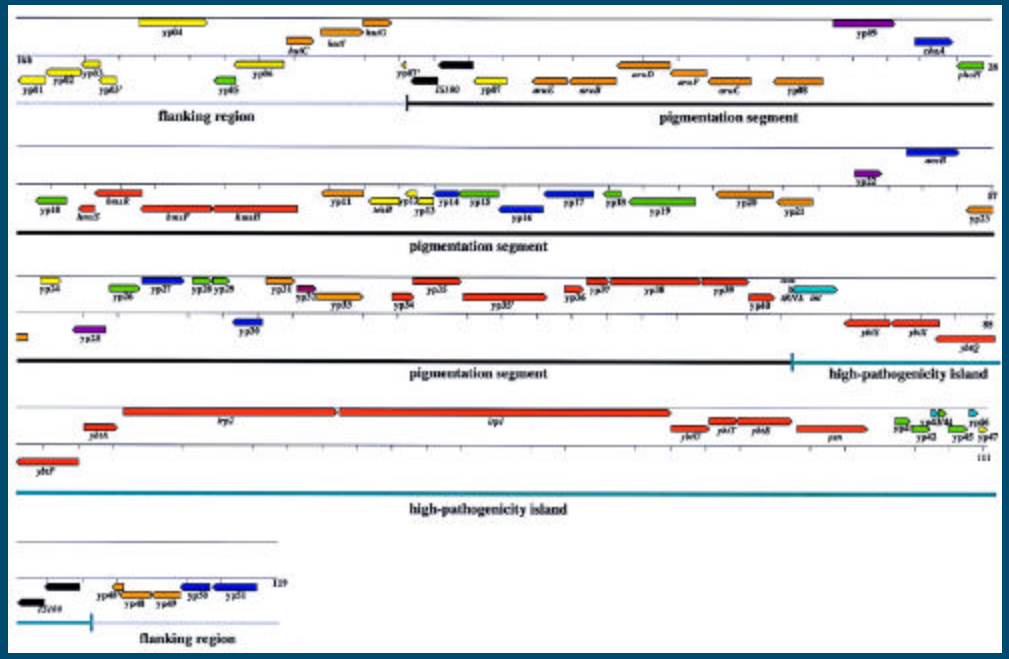
REACTION	% of recipients reporting reactions	
	After first dose (n=67)	After second dose (n=59)
LOCAL		
Tenderness	71.6	18.6
Decreased arm motion	11.9	1.7
Erythema	4.5	0
Warmth	3.0	1.7
Edema	1.5	0
SYSTEMIC		
Headache	19.4	6.8
Nausea	13.4	3.4
Malaise	10.4	5.1
Dizziness	6.0	0
Chills	4.5	3.4
Joint pain	4.5	0
Muscle pain	4.5	0
Anorexia	1.5	0
Diarrhea	1.5	0
Vomiting	1.5	0

Live attenuated vaccines

- Used since 1908, mainly in FSU and French Colonies
- Recommended dose 5.8 x 10^6 CFU
- MPI typically < 10 after immunisation
- EV series e.g EV76, EV Saigon, EV Madagascar



EV76 is a pigmentation mutant



- The *pgm* locus – spontaneously deleted
  - pigmentation
  - iron acquisition /siderophore production
  - virulence
- Evidence that EV76 has insertional inactivation of some genes

Reactogenicity of EV76 vaccine

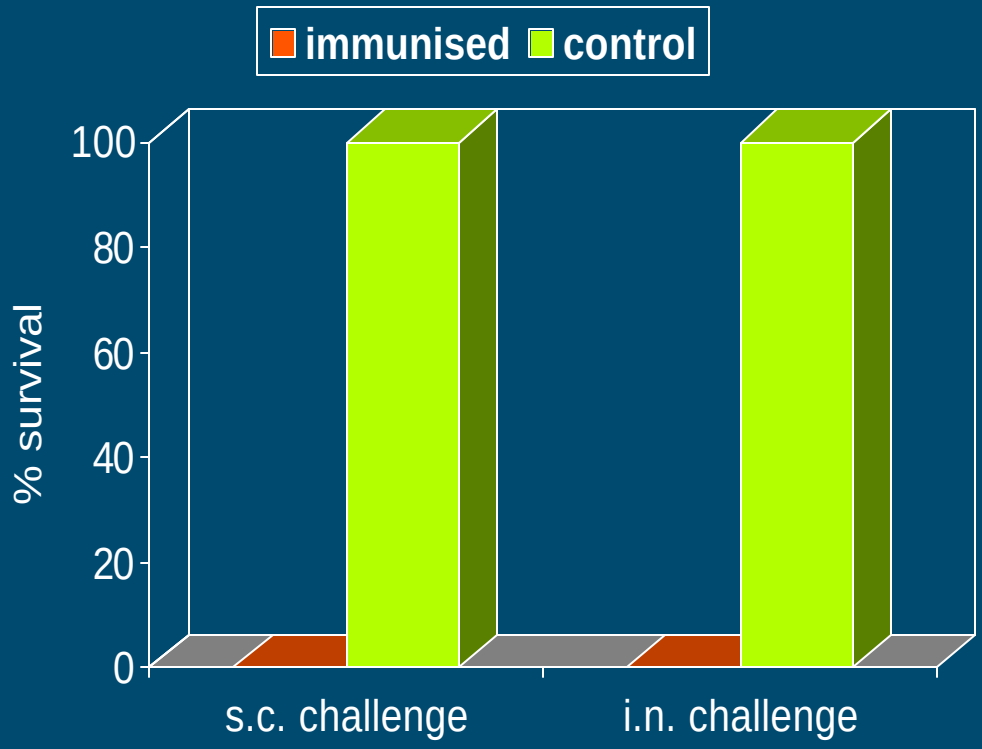
- 12/12 human volunteers developed severe systemic reactions (Meyer 1970)
  - febrile response with headache, weakness & malaise
- Frequent eythema surrounding the vaccination site – up to 15cm diameter
- Hospitalisation of some vaccinees

Efficacy of live attenuated vaccines

- data reported suggests EV vaccines protect against bubonic and pneumonic plague

i.m. immunisation

challenge with 150 MLD *Y. pestis*



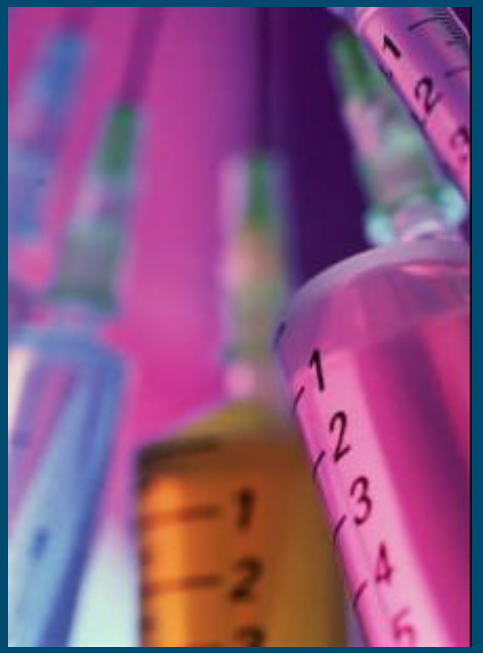
In summary

- killed whole vaccines
  - are multidose
  - are able to protect against bubonic but not pneumonic plague
  - are reactogenic
- Live vaccines
  - are single dose
  - are able to protect against bubonic and pneumonic plague
  - are highly reactogenic

An improved Vaccine

Approaches to a new vaccine

- Live attenuated mutant
- Sub-unit vaccine
- Naked DNA vaccine
- Vector delivered vaccine

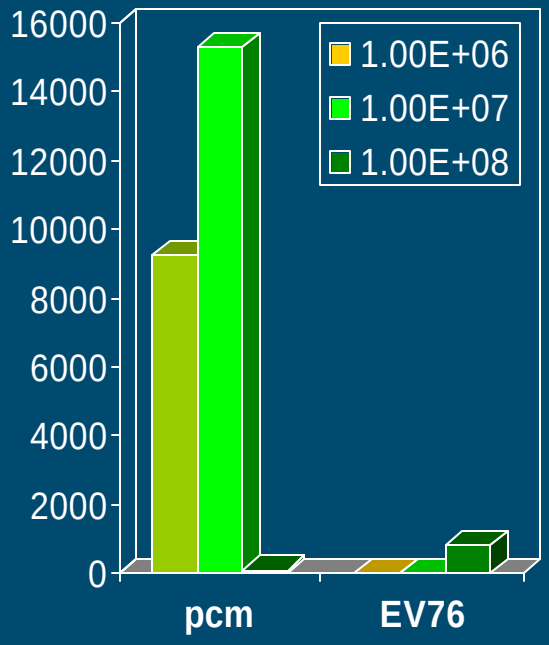


Virulence and protection afforded by *Y. pestis* mutants

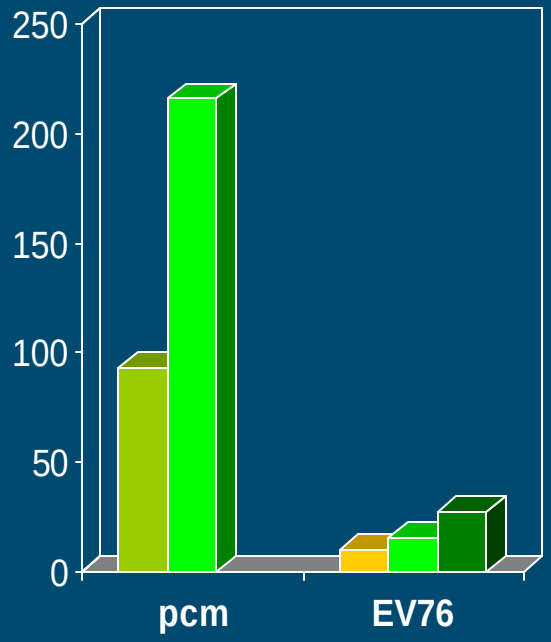
	MLD (mouse)	ttd (hr)	MLD (g. pig)	ttd (hr)	protection against subsequent challenge
WT	1	108	2	ND	
aroA	2	159	>26	ND	yes, in guinea pig
phoP	75	221	ND	ND	yes
htrA	30	192	ND	ND	ND
pcm	> 10E7				yes
dam	2300				yes > 7,500 MLD

A *pcm* mutant is a candidate live vaccine

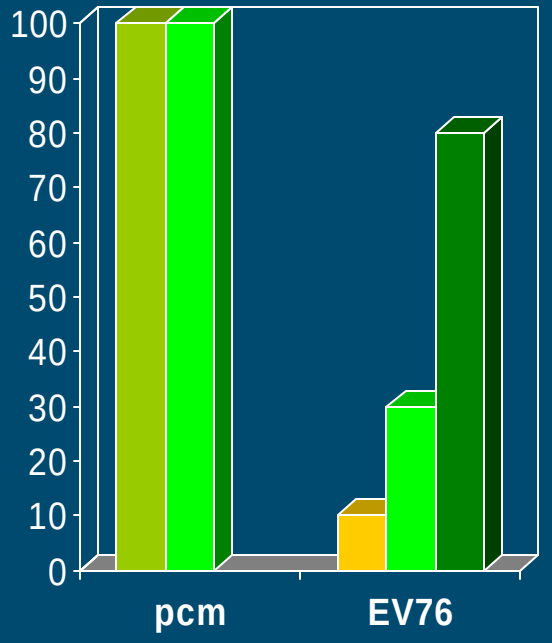
F1-antibody



V-antibody



protection (10E5 challenge)



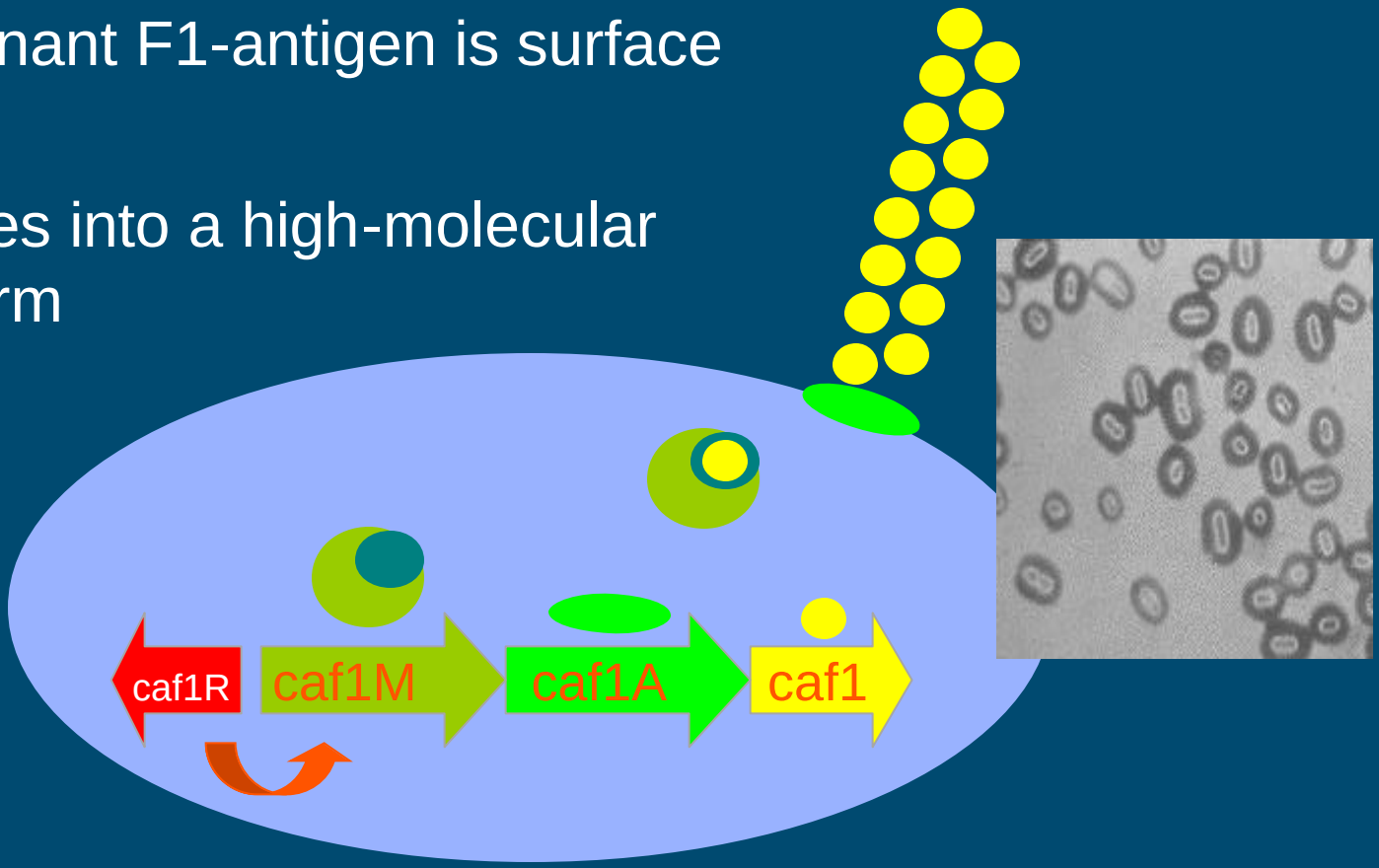
Flashner et al., (2004) Infect. Immun. 72:908-915

Sub-units evaluated as vaccine antigens

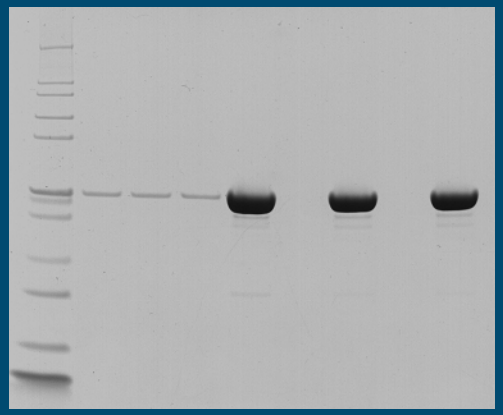
Sub-unit	Function	Immunogenic	Protective efficacy (bubonic / peumonic model)
Pla	surface plasminogen activator protease	Y	Not tested
pH6 antigen	putative surface adhesin	Y	bubonic – not protective
LPS	lipopolysaccharide	Y	bubonic – not protective
F1 antigen	surface capsule	Y	bubonic & pneumonic – protective
YopD	type III system – translocation Yop	Y	bubonic –partially protective
YopH	type III system – PTPase effector Yop	Y	bubonic – not protective
YopE	type III system – cytotoxin effector Yop	Y	bubonic – not protective
YopN	type III system – regulates Yop release ?	Y	bubonic – not protective
YopK	type III system – regulates Yop release ?	Y	bubonic – not protective
YopM	type III system – effector Yop	Y	bubonic – not protective
Ypk A	type III system – Ser/Thr kinase effector Yop	Y	bubonic – delayed time to death
V antigen	type III system – part of the injectosome ?	Y	bubonic & pneumonic – protective

The *caf* operon in *E. coli* directs the expression of recombinant F1-antigen

- Recombinant F1-antigen is surface located
- Assembles into a high-molecular weight form



Expression of recombinant LcrV

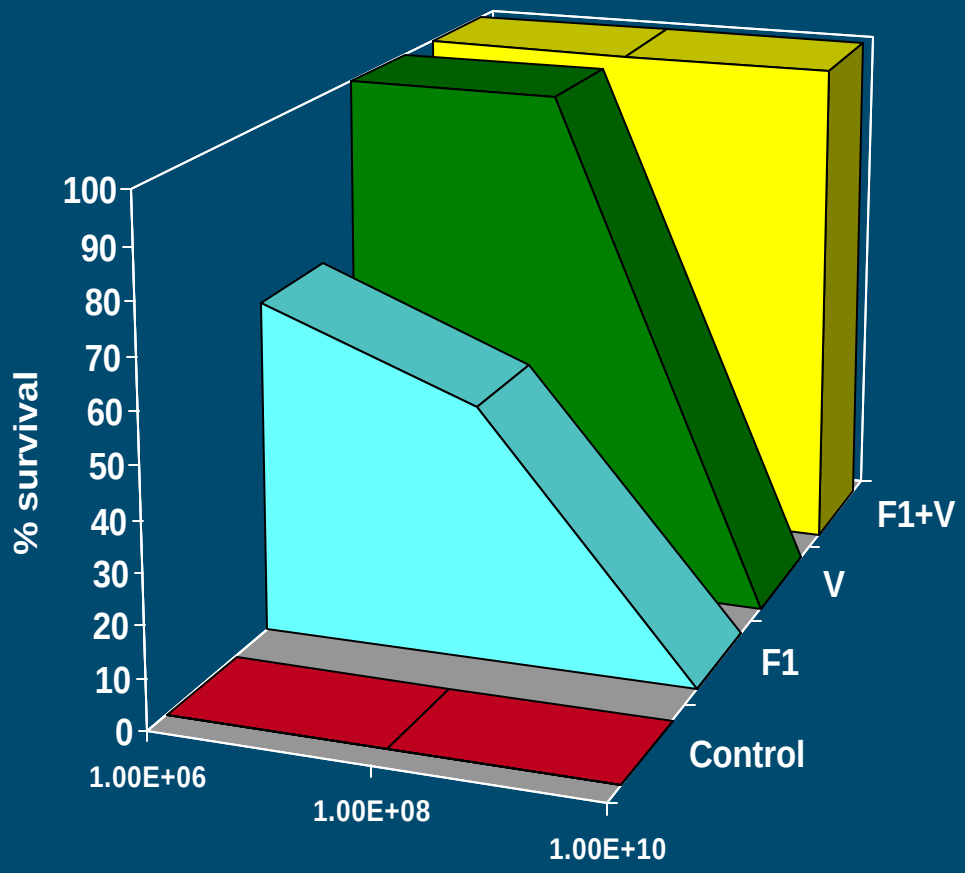


- Expressed in *E. coli* as a GST-fusion protein
- Cleaved from GST carrier using PreScission™ protease

rV GPLGSPG**IRAYEQNPQHFIEDLEKVRVE**…….

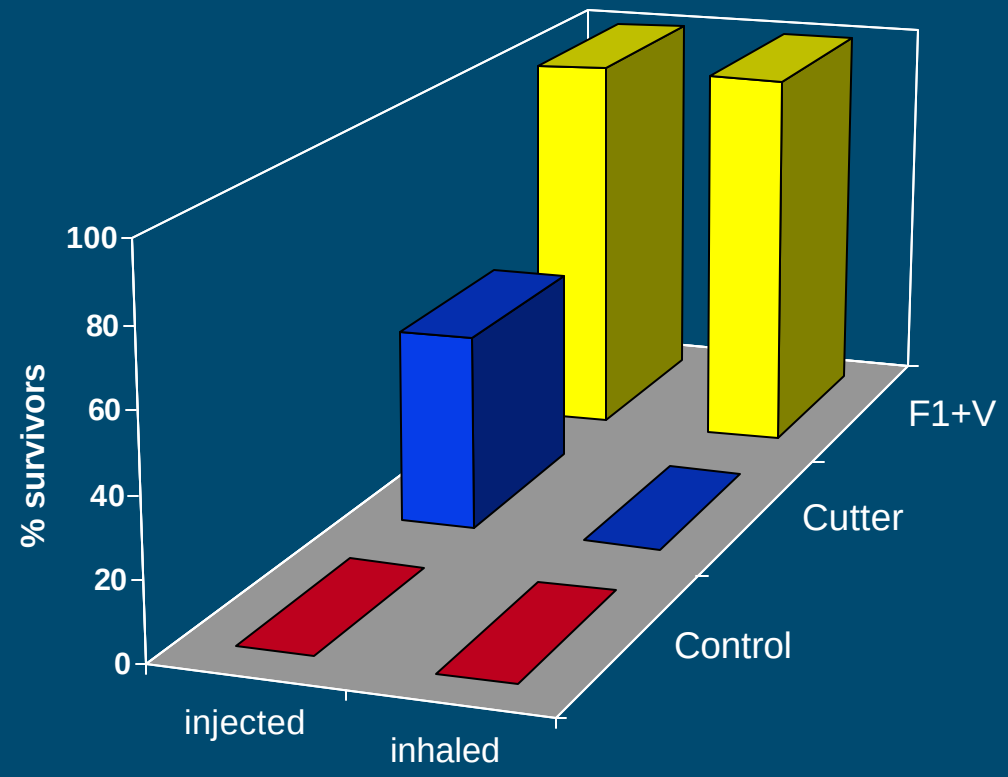
nativeV **IRAYEQNPQHFIEDLEKVRVE**…….

Protection afforded by F1+V vaccine against *Y. pestis*



- F1 or V alone induce protection against low challenge doses
- F1+V together induce "solid" protection

Protection afforded by F1+V antigens against 10^5 cfu *Y. pestis* strain GB



- Protection demonstrated against s.c. or inhalation challenge with
  - *Y. pestis* CO92
  - *Y. pestis* Java 9 (F1-)

Recombinant plague vaccine

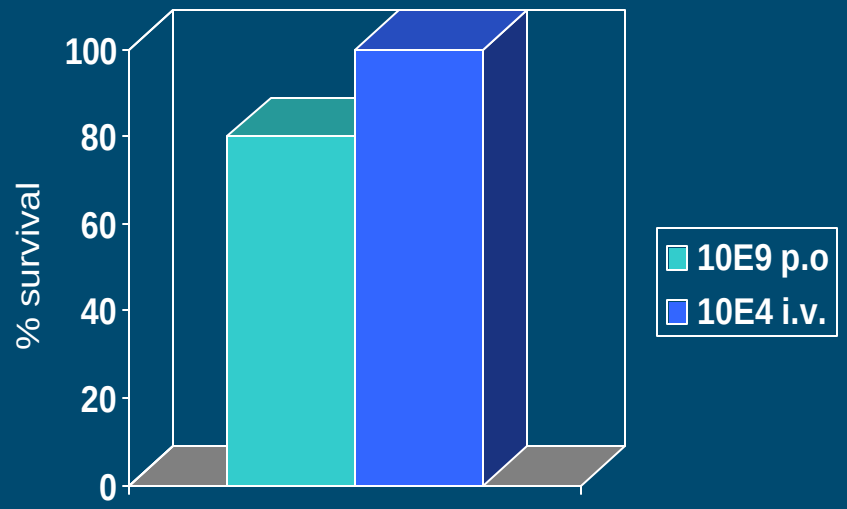
- alhydrogel adjuvant
- 2 dose schedule - days 1 and 21
- each vial contains 40µg of F1-antigen and 40µg of V-antigen

Are there additional protective antigens ?

- immunisation with a pCD1 cured *dam* mutant strain of *Y. pseudotuberculosis* protects against plague

- This points towards protective antigens other than F1 and V

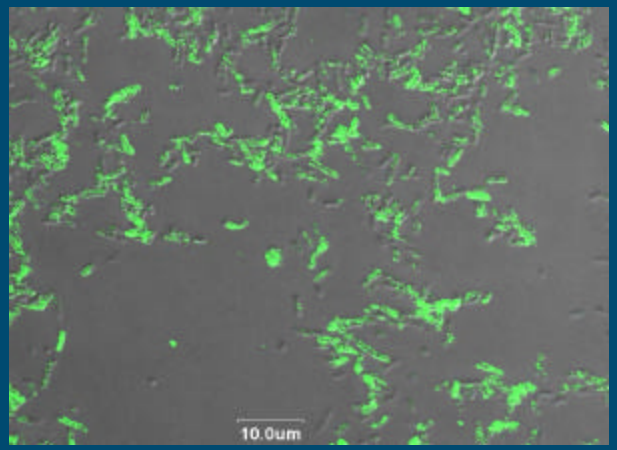
s.c. challenge with 100 MLD *Y. pestis*

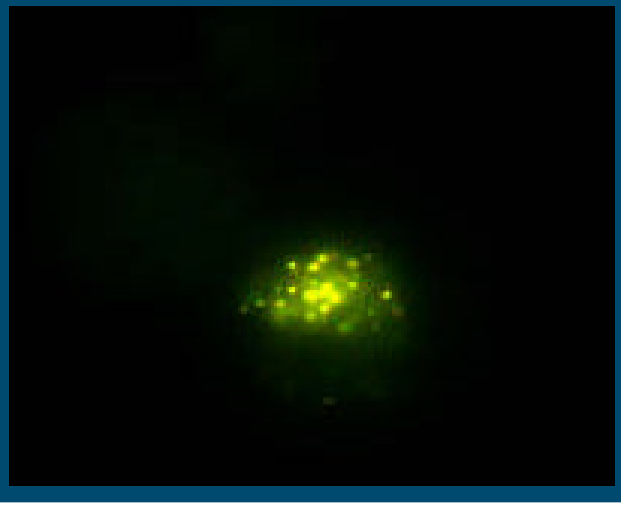


Third Generation vaccines

Salmonella enterica serovar *typhi* expressing F1-antigen

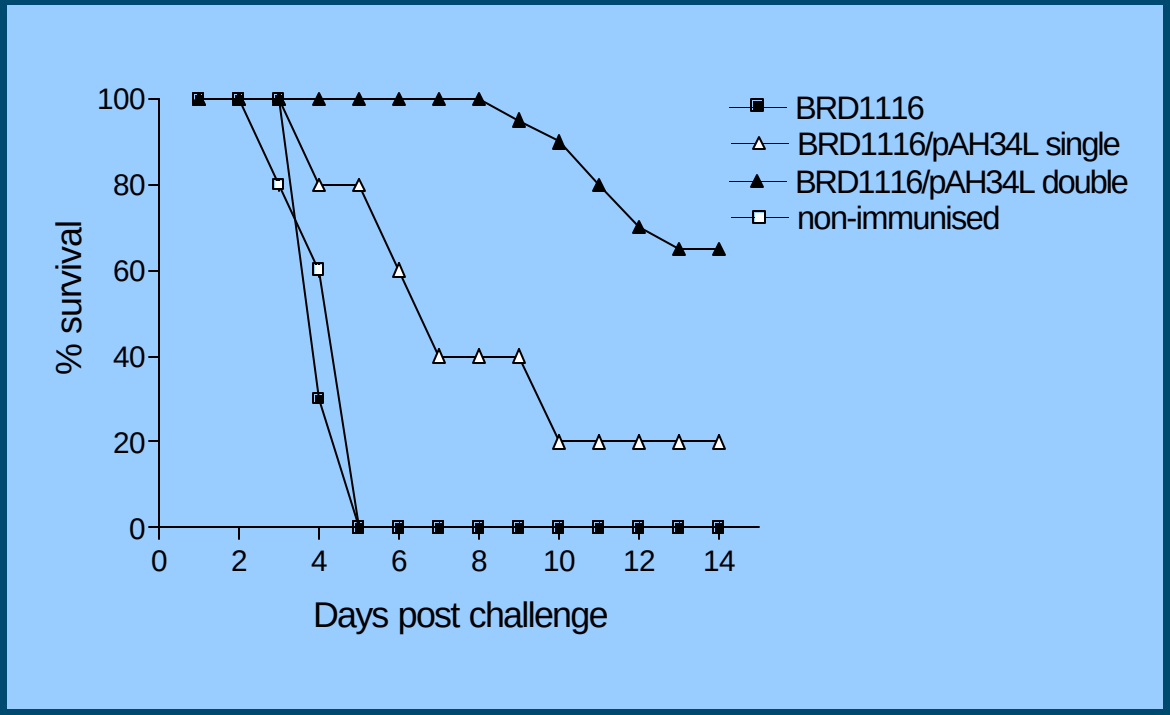
- expression in BRD1116 (*aroA*, *aroC*, *htrA*)
- Expression demonstrated within macrophages





Immunised mice are protected

- i.n. immunisation with 10^8 cfu of *S. enterica*
- s.c. challenge with 113 MLD of *Y. pestis*



Naked DNA vaccines

- naked DNA vaccines encoding F1- or V-antigens protect against plague
- multiple doses of the naked DNA are required
- in some cases prime-boost strategies are required to elicit protection

Summary

- Existing KWC or live vaccines have significant limitations
- Effective and safe live vaccines appear feasible (but are they acceptable)
- An F1-V vaccine is effective and appears to be safe
- There may be additional protective antigens
- Orally or intranasally delivered vaccines appear to be feasible

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